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Abstract: One in five adults following physical activity guidelines are reported not demonstrating any improvement in cardiorespiratory fitness (CRF). Herein, we sought to establish whether CRF non-response to exercise training is dose-dependent, using a between- and within-subject study design. Seventy-eight healthy adults were divided into 5 groups ('1', '2', '3', '4' and '5') respectively comprising 1, 2, 3, 4 and 5 × 60 min exercise sessions per week but otherwise following an identical 6-week endurance training (ET) program. Non-response was defined as any change in CRF, determined by maximal incremental exercise power output (W_{max}), within the typical error of measurement ($\pm 3.96\%$). Participants classified as non-responders after the ET intervention completed a successive 6-week ET period including 2 additional exercise sessions per week. Maximal oxygen consumption (VO_{2max}), haematology and muscle biopsies were assessed prior to and after each ET period. After the first ET period, W_{max} increased ($P < 0.05$) in groups '2', '3', '4' and '5', but not '1'. In groups '1', '2', '3', '4' and '5', 69%, 40%, 29%, 0% and 0% of individuals, respectively, were non-responders. After the second ET period, non-response was eliminated in all individuals. The change in VO_{2max} with exercise training independently determined W_{max} response (partial correlation coefficient ($r_{partial}$) 0.74, $P < 0.001$). In turn, total hemoglobin mass was the strongest independent determinant of VO_{2max} ($r_{partial} = 0.49$, $P < 0.001$). In conclusion, individual CRF non-response to exercise training is abolished by increasing the dose of exercise and primarily a function of haematological adaptations in oxygen-carrying capacity. This article is protected by copyright. All rights reserved.

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Refuting the myth of non-response to exercise training: 'non-responders' do respond to higher dose of training

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Key points

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- The prevalence of cardiorespiratory fitness (CRF) non-response gradually declines in healthy individuals exercising 60, 120, 180, 240 or 300 min per week for 6 weeks.
- Following a successive identical 6-week training period but comprising 120 min of additional exercise per week, CRF non-response is universally abolished.
- The magnitude of CRF improvement is primarily attributed to changes in hemoglobin mass.
- The potential for CRF improvement may be present and unveiled with appropriate exercise training stimuli in healthy individuals without exception.

Abstract One in five adults following physical activity guidelines are reported not demonstrating any improvement in cardiorespiratory fitness (CRF). Herein, we sought to establish whether CRF non-response to exercise training is dose-dependent, using a between- and within-subject study design. Seventy-eight healthy adults were divided into 5 groups ('1', '2', '3', '4' and '5') respectively comprising 1, 2, 3, 4 and 5×60 min exercise sessions per week but otherwise following an identical 6-week endurance training (ET) program. Non-response was defined as any change in CRF, determined by maximal incremental exercise power output (W_{\max}), within the typical error of measurement ($\pm 3.96\%$). Participants classified as non-responders after the ET intervention completed a successive 6-week ET period including 2 additional exercise sessions per week. Maximal oxygen consumption ($VO_{2\max}$), haematology and muscle biopsies were assessed prior to and after each ET period. After the first ET period, W_{\max} increased ($P < 0.05$) in groups '2', '3', '4' and '5', but not '1'. In groups '1', '2', '3',

'4' and '5', 69%, 40%, 29%, 0% and 0% of individuals, respectively, were non-responders. After the second ET period, non-response was eliminated in all individuals. The change in $\text{VO}_{2\text{max}}$ with exercise training independently determined W_{max} response (partial correlation coefficient ($r_{\text{partial}} \geq 0.74$, $P < 0.001$). In turn, total hemoglobin mass was the strongest independent determinant of $\text{VO}_{2\text{max}}$ ($r_{\text{partial}} = 0.49$, $P < 0.001$). In conclusion, individual CRF non-response to exercise training is abolished by increasing the dose of exercise and primarily a function of haematological adaptations in oxygen-carrying capacity.

Abbreviations BV, blood volume; CO, carbon monoxide; CRF, cardiorespiratory fitness; ET, endurance training; [Hb], haemoglobin concentration; Hb_{mass} , haemoglobin mass; Hct, haematocrit; HR_{max} , maximal heart rate; Mito_{VD} , mitochondrial volume density; PV, plasma volume; RBCV, red blood cell volume; TE, typical error of measurement; $\text{VO}_{2\text{max}}$, maximal oxygen consumption; W_{max} , maximal power output.

Introduction

Primary sources regarding the notion of physical activity as a means to preserve health emerged along with the inception of scientific medicine in ancient Greece (ACSM, 2013). More than 2 millennia later, outstanding health benefits associated with physical activity have been demonstrated by modern experimental, epidemiological and clinical sciences (Morris *et al.*, 1953; Lee *et al.*, 1995; Church *et al.*, 2007; Wen *et al.*, 2011; Pedersen & Saltin, 2015). Daily vigorous exercise decreases cardiovascular, metabolic and all-cause mortality by 40 to 70 %, irrespective of age, gender and presence of disease (Wen *et al.*, 2011). Many of these benefits are closely associated with improvements in cardiorespiratory fitness (CRF),

as determined by maximal oxygen consumption ($\text{VO}_{2\text{max}}$) or incremental exercise power output (W_{max}), independent of traditional risk factors (Joyner & Green, 2009; Lee *et al.*, 2010). CRF is thus considered a key surrogate endpoint of exercise training interventions in healthy and diseased individuals (Laukkanen *et al.*, 2001; Kodama *et al.*, 2009).

Among exercise training modalities, CRF is particularly responsive to endurance training (ET). $\text{VO}_{2\text{max}}$ and W_{max} commonly increase by an average of ~3-35 % at the group level with regular ET in a dose (exercise duration \times intensity)-dependent manner (Wenger & Bell, 1986; Church *et al.*, 2007; Helgerud *et al.*, 2007; Bouchard *et al.*, 2011b; Bacon *et al.*, 2013; Jacobs *et al.*, 2013; Bonne *et al.*, 2014; Montero *et al.*, 2015a). $\text{VO}_{2\text{max}}$ and W_{max} gains induced by a given dose of supervised ET are however not uniform at the individual level, one third of measurements being dispersed more than one standard deviation from the mean (Cohen & Holliday, 1979; Lortie *et al.*, 1984; Bouchard *et al.*, 2011b; Bacon *et al.*, 2013). Importantly, some individuals do seemingly not demonstrate any improvement in $\text{VO}_{2\text{max}}$ with ET according to current dose recommendations (150 minutes of moderate endurance exercise per week) (Bouchard *et al.*, 2011b; Garber *et al.*, 2011). The prevalence of $\text{VO}_{2\text{max}}$ non-responders has been reported to reach up to 20 % among healthy individuals (Timmons *et al.*, 2010), raising compelling challenges to personalized preventive medicine (Buford *et al.*, 2013).

As a first step, it is fundamental to understand which factors contribute to interindividual differences in CRF trainability. In this regard, much attention has been paid to genetic determinants (Bouchard *et al.*, 1999; Timmons *et al.*, 2010; Bouchard *et al.*, 2011a; Bouchard *et al.*, 2011b; Bouchard, 2012; Sarzynski *et al.*, 2016). Herein, 21 single-nucleotide polymorphisms were found to explain up to 50 % of the $\text{VO}_{2\text{max}}$ individual response to 5 months of moderate ET in a cohort of 473 sedentary adults from the HERITAGE Family Study (HERITAGE henceforward) (Bouchard *et al.*, 2011b). Approximately 8 % of these individuals increased $\text{VO}_{2\text{max}}$ by 28 % or more, whereas ~7 % demonstrated a change in $\text{VO}_{2\text{max}}$ of only 4 % or less (Bouchard *et al.*, 2011b). While these findings have to be replicated, the notion has spread that CRF trainability is essentially a matter of fate (Roth, 2008; Bouchard, 2012; Mosley, 2012). Yet, it is uncertain whether HERITAGE outcomes can be extrapolated to at-risk or diseased populations and above all, it remains to be established whether or to what extent CRF non-response is dose-dependent.

The influence of ET dose on CRF non-response was recently examined in 3 groups of obese adults exposed to 3 distinct levels of exercise amount and intensity for 6 months (Ross *et al.*, 2015). CRF non-response, as defined by any change in $\text{VO}_{2\text{max}}$ lower than the typical error of measurement (TE), was observed in 39, 18 and 0 % of obese individuals respectively randomized to low-amount ($180\text{-}300 \text{ kcal}\cdot\text{session}^{-1}$)/low-intensity (50 % $\text{VO}_{2\text{max}}$), high-amount ($360\text{-}600 \text{ kcal}\cdot\text{session}^{-1}$)/low-intensity (50 % $\text{VO}_{2\text{max}}$) or high-amount ($360\text{-}600 \text{ kcal}\cdot\text{session}^{-1}$)/high-intensity (75 % $\text{VO}_{2\text{max}}$) ET,

all including 4-5 exercise sessions per week (Ross *et al.*, 2015). A similar decline in the prevalence of $\text{VO}_{2\text{max}}$ non-responders was previously demonstrated in postmenopausal women allocated to 4-5 exercise sessions requiring 4, 8 or 12 $\text{kcal}\cdot\text{kg}^{-1}$ per week during 6 months (Sisson *et al.*, 2009). These studies therefore suggest that CRF non-response might ultimately be the result of inadequate exercise stimuli. Nonetheless, it should be noted that only individuals who adhered to at least 90% of the prescribed exercise sessions were included in these analyses (Sisson *et al.*, 2009; Ross *et al.*, 2015). It thus cannot be discarded that non-responders were primarily excluded by study design. To unequivocally overcome this issue, CRF non-responders should first be identified and then subjected to higher dose ET (Mann *et al.*, 2014). This may be ideally addressed using a repeated ET intervention, which likewise would limit the influence of within-subject response variability, a well-known source of confounding in the clinical arena but uncertain concerning exercise training studies (Senn *et al.*, 2011; Hecksteden *et al.*, 2015).

Therefore, we assessed the response of CRF to ET in healthy young individuals divided into 5 groups comprising 1, 2, 3, 4 or 5 \times 60 min exercise sessions) per week during 6 weeks. We used this relatively short length of ET in order to secure a high prevalence of CRF non-responders while still provoking extensive physiological responses at the group level (Montero *et al.*, 2015a). Likewise, W_{max} was chosen as the primary CRF outcome because its TE, as measured in our laboratory, is lower compared with that frequently reported for $\text{VO}_{2\text{max}}$ ($\sim 5\%$) (Shephard *et al.*, 2004);

indeed, the relatively large contribution of test–retest errors in $VO_{2\max}$ to the individual response to ET has been a matter of controversy concerning seminal studies in the current topic (Shephard *et al.*, 2004; Hecksteden *et al.*, 2015).

Individuals identified as non-responders subsequently completed an identical 6-week ET period but including 2 additional exercise sessions per week, thereby augmenting the ET dose dispensed. In addition, $VO_{2\max}$ as well as potential haematological and skeletal muscle determinants of W_{\max} were assessed prior to and after each ET period with the intent to determine physiological relevant parameters that may explain variations in trainability.

Methods

Participants

Seventy-eight healthy young males volunteers (age = 26.2 ± 3.4 yr, height = 180.8 ± 4.3 cm) were recruited to participate in the study. To this end, ads were placed at the university campus looking for untrained individuals. As inclusion criteria, individuals had to be to males between 18-35 years old not participating in organized sports, non-smokers and medication free. Cycling and walking for commuting purposes was allowed. The protocol was approved by the local ethical committee (EK 2011-N-51) and conducted in accordance with the Declaration of Helsinki. Prior to the start of the experiments, informed oral and written consents were obtained from all participants.

Experimental design

All individuals underwent a first period of supervised training for 6 weeks consisting of 60 min sessions of cycle ergometer exercise. Individuals could chose to participate in 5 groups differing in the number of exercise sessions per week. Groups '1', '2', '3', '4' and '5' performed 1, 2, 3, 4 and 5 exercise sessions per week, respectively, corresponding to 60, 120, 180, 240 and 300 total min per week. Four different intensity profiles, comprising moderate continuous exercise and high-intensity intervals were alternated to facilitate participant motivation and compliance, as detailed previously (Robach *et al.*, 2014). Profile 1 consisted of a steady-state exercise, i.e. 60 min at 65 % of peak power output (W_{\max}) attained with the incremental exercise test. Profile 2 started with 11.25 min at 65 % of W_{\max} followed by 0.5^{130} , 1.25^{50} , 0.5^{130} , 1^{50} , 11.25^{65} , 0.5^{130} , 1.25^{50} , 0.5^{130} , 1.25^{50} , 0.5^{130} , 1^{50} , 11.25^{65} , 0.5^{130} , 1.25^{50} , 0.5^{130} , 1.25^{50} , 0.5^{130} , 1^{50} and 11.25^{65} . Profile 3 started with 3 min at 50 % of W_{\max} followed by 3^{60} , 3^{65} , 3^{70} , 3^{75} , 3^{70} , 3^{65} , 3^{60} , 3^{50} , 3^{65} , 3^{50} , 3^{60} , 3^{65} , 3^{70} , 3^{75} , 3^{70} , 3^{65} , 3^{60} , 3^{50} and 3^{65} . Profile 4 started with 6 min at 65 % of W_{\max} followed by 4^{75} , 6^{65} , 4^{75} , 6^{65} , 4^{75} , 6^{65} , 4^{75} , 6^{65} , 4^{75} , 6^{65} and 4^{75} . Each profile had an average exercise intensity of 65% of W_{\max} for 60 min. Workloads were calculated from individual W_{\max} determined during the incremental exercise test at baseline. CRF non-response was determined using the TE (Hopkins, 2000). TE is a measure of technological error and day-to-day biological variation. TE was calculated by the standard error of within-subject standard deviation from duplicate W_{\max}

assessments, and expressed as a percent of the individual's mean W_{\max} (i.e., in the form of a coefficient of variation). Any percentage change in W_{\max} not beyond $\pm 1 \times \%TE$ was considered a non-response. Based on repeated testing at baseline in all individuals, CRF non-response was calculated to be $\pm 3.96 \%$ for W_{\max} . The average of duplicate W_{\max} assessments was considered the baseline W_{\max} for each individual. Less than 7 days after the end of the initial ET period, non-responders in each exercise group started a second 6-week ET program identical to the former but adding 2 exercise sessions per week. The measures described below were determined prior to and after the first ET period as well as following the second ET period.

Experimental measures

Incremental exercise test. W_{\max} and $VO_{2\max}$ were determined on an electronically braked bicycle ergometer (Monark, Sweden) with continuous measurements of VO_2 using an online gas collection system (Innocor M400, Innovision, Denmark). The test started with a warm-up period of 5 min at 50-150 W workloads. Thereafter, the workload was increased by 30 W every 60- s until exhaustion. The gas analysers and the flowmeter of the applied spirometer were calibrated prior to each test. Breath-by-breath values were averaged over 30 s. The highest average value was taken as the $VO_{2\max}$ provided that standard criteria were fulfilled (American Thoracic Society,

2003). W_{\max} was calculated as $W_{\text{compl}} + 30 \cdot (t/60)$; W_{compl} is the last fully completed workload and t is the number of seconds in the final workload.

Blood. Haemoglobin mass (Hb_{mass}) was measured as previously described (Siebenmann *et al.*, 2015), using a modified version of the carbon monoxide (CO) re-breathing technique (Burge & Skinner, 1995). All individuals rested for 20 min in a semi-recumbent position before each measurement. Thereafter, 2 ml of blood was sampled from an antecubital vein via a 20-G venflon (BD, USA) and analysed immediately in quadruplicate for (i) percent carboxyhaemoglobin and Hb concentration ($[Hb]$) using a hemoximeter (ABL800, Radiometer, Denmark), and (ii) haematocrit (Hct) with the micromethod (4 min at 13500 rpm). Subsequently, the subject breathed 100 % oxygen for 4 min to flush the nitrogen from the airways. After closing the oxygen input, a bolus 1.5 ml/kg of 99.997 % chemically pure CO (CO N47, Air Liquide, France) was administrated into the breathing circuit. The subjects rebreathed this gas mixture for 10 min. Then, an additional 2 ml blood sample was obtained and analysed in quadruplicate. The change in percent carboxyhaemoglobin was used to calculate Hb_{mass} , taking into account the amount of CO that remained in the rebreathing circuit at the end of the procedure (2.2 %). (Burge & Skinner, 1995) Total red blood cell volume (RBCV), blood volume (BV) and plasma volume (PV) were derived from measures of Hb_{mass} and Hct (Burge & Skinner, 1995).

Skeletal muscle biopsies. Using the Bergström technique (Bergstrom, 1962) with a needle modified for suction, skeletal muscle biopsies from *m. vastus lateralis* were obtained under local anaesthetics while the subject was at rest with a minimum of 24 h following the last exercise training bout. The biopsy specimen was dissected free of fat and connective tissue, divided into sections and immediately prepared for analysis as stated below.

Mitochondrial volume density (Mito_{VD}). Four 1 mm³ pieces of each muscle biopsy were fixed in 2.5% glutaraldehyde at room temperature and processed according to standard electron-microscopy protocols. TEM images were obtained in a FEI Tecnai G2 Spirit electron microscope (FEI, USA) with an Orius SC1000 CCD camera (Gatan, USA) and interfaced with the TEM User software from FEI (FEI, USA). Two hundred and sixteen images per biopsy were acquired in a random systematic order from 24 meshes distributed on 8 grids from 4 blocks. The Cavalieri feature in the Stereo-Investigator software (MBF Bioscience, USA) was used to estimate mitochondrial volume density (Mito_{VD}) by point counting (West, 2012). The grid spacing was 1 µm along both x- and y-axis. Mitochondria boundaries were recognized at the 8200x magnification. Each point was assigned as either mitochondria, muscle or "nothing". Mito_{VD} was expressed as the percentage of muscle tissue occupied by mitochondria. Mito_{VD} was not assessed after the second training period.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (SPSS, Chicago, USA). Data were tested for normal distribution with the Kolmogorov-Smirnov test and for homogeneity of variances with Levene's test. One-way ANOVA with Bonferroni correction for multiple comparisons was used to compare continuous baseline variables among groups. To examine the impact of the frequency of exercise sessions on the effects of exercise training, a one-way ANOVA was applied including baseline values as covariates and Bonferroni correction for multiple comparisons. When F was significant in the ANOVA, planned pair-wise specific comparisons were carried out. Effects of exercise training on studied variables in each group were assessed by paired *t*-tests. The proportion of CRF non-responders was compared among groups with the Chi-Square test. Finally, multiple regression analysis was used to identify variables independently associated with absolute changes (post training minus pre training, Δ) in W_{\max} or $VO_{2\max}$, including first and second ET periods. Variables significantly associated with ΔW_{\max} or $\Delta VO_{2\max}$ in univariate analyses, as determined by Pearson's correlation coefficients, were entered into the regression model as independent variables. In case of high correlation between independent variables, each of these were separately entered into the regression model in order to avoid high multicollinearity (variance inflation factor >10). A two-tailed *P*-value less than 0.05 was considered significant. Data are reported as mean (\pm SD) unless otherwise stated.

Results

Baseline

Characteristics of study groups prior to ET are summarized in Table 1. Body weight, haematological (Hct, Hb, Hb_{mass}, PV, RBCV, BV), skeletal muscle Mito_{VD} and VO_{2max} were similar between groups ($P > 0.05$). The only difference between groups was observed with regard to W_{max}, which was higher in group '5' compared with group '3' either as expressed in absolute (W) or relative (W·kg⁻¹) units ($P < 0.05$).

First exercise training period

Table 2 presents absolute changes (Δ) after the first ET period in each group. A decrease in body weight with training was only observed in groups '4' and '5' ($P < 0.05$). Mito_{VD} increased in all groups ($P < 0.05$). Hb_{mass}, RBCV, BV, VO_{2max} and W_{max} increased in groups all groups ($P < 0.05$) except for group '1'. With respect to the comparisons of Δ among groups, Δ body weight was lower (negative) in group '5' compared with groups '1', '2' and '3' ($P < 0.05$). Moreover, Δ Mito_{VD} was higher in groups '4' and '5' versus groups '1' and '2' ($P < 0.05$). Δ Hb_{mass}, Δ RBCV, Δ BV and Δ VO_{2max} were higher in group '5' compared with any other group ($P < 0.05$). Δ W_{max} was higher in groups '4' and '5' versus groups '1', '2' and '3' ($P < 0.05$). Analyses with percentage (instead of Δ) changes and/or without covariate adjustment gave similar results. Figure 1 illustrates individual percentage changes in W_{max} after the first ET period. The prevalence of W_{max} non-response was 69 % (11 of 16 individuals), 40 % (6 of 15), 29 % (4 of 14), 0 % (0 out of 17) and 0 % (0 out of 16) for '1', '2', '3', '4' and '5' groups, respectively ($P < 0.001$). The average percentage change in W_{max} of non-

responder individuals was -1 %, -1 % and 2 % for '1', '2', '3' groups, respectively.

Whilst not measured in the current study, provided a TE for $\text{VO}_{2\text{max}}$ of 5 % (Shephard *et al.*, 2004), the prevalence of $\text{VO}_{2\text{max}}$ non-response would be 81 % (13 of 16 individuals), 47 % (7 of 15), 50 % (7 of 14), 18 % (3 out of 17) and 0 % (0 out of 16) for '1', '2', '3', '4' and '5' groups, respectively.

Second exercise training period

Non-responders to the first ET period subsequently performed a second ET intervention including 2 additional training sessions per week. Paired comparisons were performed after the second ET period versus baseline (prior to the first ET period) in non-responder individuals, thus assessing the total impact of both training periods in each group ($n = 11$, '1 + 2'; $n = 6$, '2 + 2'; $n = 4$, '3 + 2'). Body weight decreased in group '2 + 2' ($P < 0.05$). Hb_{mass} , RBCV, BV, $\text{VO}_{2\text{max}}$ and W_{max} increased in all groups ('1 + 2', '2 + 2', '3 + 2') ($P < 0.05$). Mito_{VD} was not assessed after the second ET period. Analyses with percentage (instead of Δ) changes and/or without covariate adjustment gave similar results. Figure 2 displays individual percentage changes in W_{max} after the first and second ET periods for non-responders individuals. Non-response was not observed after the second ET period in any individual.

Determinants of changes in ΔW_{max} and $\Delta \text{VO}_{2\text{max}}$

In univariate analysis, $\Delta \text{VO}_{2\text{max}}$ ($r = 0.85$, $P < 0.001$), $\Delta \text{Hb}_{\text{mass}}$ ($r = 0.64$, $P < 0.001$), ΔRBCV ($r = 0.62$, $P < 0.001$), ΔBV ($r = 0.53$, $P < 0.001$), ΔPV ($r = 0.42$, $P < 0.001$) and $\Delta \text{Mito}_{\text{VD}}$ ($r = 0.26$, $P = 0.043$) and were positively whereas $\Delta \text{body weight}$ ($r = -0.29$, P

= 0.003) negatively associated with ΔW_{\max} . Moreover, ΔHb_{mass} ($r = 0.64$, $P < 0.001$), $\Delta RBCV$ ($r = 0.62$, $P < 0.001$), ΔBV ($r = 0.52$, $P < 0.001$) and ΔPV ($r = 0.42$, $P < 0.001$) were positively and $\Delta \text{body weight}$ ($r = -0.25$, $P = 0.012$) negatively associated with $\Delta VO_{2\max}$. Accordingly, ΔHb_{mass} , $\Delta RBCV$, ΔPV , ΔBV , $\Delta \text{Mito}_{\text{VD}}$, $\Delta VO_{2\max}$ and $\Delta \text{body weight}$ were entered into the regression model as potential independent determinants of ΔW_{\max} (Table 3), while ΔHb_{mass} , $\Delta RBCV$, ΔPV , ΔBV and $\Delta \text{body weight}$ were entered as potential independent determinants of $\Delta VO_{2\max}$ (Table 4). $\Delta VO_{2\max}$ remained the only variable independently associated with ΔW_{\max} ($\beta = 0.08$, $r_{\text{partial}} \geq 0.74$, $P < 0.001$). In turn, ΔHb_{mass} was the strongest independent determinant $\Delta VO_{2\max}$ ($\beta = 3.26$, $r_{\text{partial}} = 0.49$, $P < 0.001$).

Discussion

The present study tested the prevalence of CRF non-response, as defined by any change of W_{\max} not surpassing %TE, with a double 6-week ET protocol in 78 healthy individuals. The key findings are: 1) after the first ET period, the prevalence of CRF non-response was 69, 40 and 29 % in individuals training for 60, 120 or 180 min per week, respectively, and was absent (0 %) in those training for 240 or 300 min per week; 2) CRF non-response was completely eliminated following the second 6-week ET period including 2 extra 60 min sessions per week; and 3) CRF (non-)response was underlay by changes in Hb_{mass} .

While the existence of interindividual response variability to exercise training is beyond dispute, less convincing is the generalized idea that a substantial fraction

(~20 %) of individuals fail to improve CRF following intense ET (Timmons *et al.*, 2010). Supporting evidence primarily derives from HERITAGE, in which 473 Caucasian adults (51 % females) completed 3 exercise sessions per week progressively increasing their length from 30 to 50 min and intensity from 55 % to 75 % of maximal heart rate (HR_{max}), during 5 months (Bouchard *et al.*, 1995; Timmons *et al.*, 2010). The HERITAGE ET program thus reached the low end of current recommendations as regards exercise time per week (150 min) and did not comprise high intensity exercise ($\geq 80\%$ HR_{max}) (Garber *et al.*, 2011). Of note, since exercise intensity was controlled by heart rate monitoring, interindividual fluctuations in training workload did occur and partly explained VO_{2max} responses to ET in HERITAGE (Sarzynski *et al.*, 2016). Most importantly, meta-analytical evidence encompassing research performed during the last 4 decades suggests that higher doses of ET than those prescribed in HERITAGE can generate substantial improvements in VO_{2max} in nearly all young and middle-aged adults (Bacon *et al.*, 2013). Likewise, recent studies have observed gradual decreases in the number of CRF non-responders with increasing doses of ET in individuals who completed $\geq 90\%$ of the exercise sessions prescribed (Sisson *et al.*, 2009; Ross *et al.*, 2015). A key limitation of these studies, however, is the potential presence of CRF non-responders in individuals who dropped out or failed to complete the minimum number of exercise sessions. Definitive proof that CRF non-responders may benefit from higher ET doses can only

be provided by within-subject study designs (Mann *et al.*, 2014; Hecksteden *et al.*, 2015), as originally applied in the current study and discussed hereunder.

We sought to determine CRF trainability with a repeated 6-week ET intervention combining moderate continuous with high intensity exercise intervals in 60 min sessions. In the first ET period, individuals from 5 groups only differing in the number of sessions (1 to 5) and thus exercise dose per week were compared. In more than 20 % of individuals allocated to 1 (60 min), 2 (120 min) or 3 (180 min) sessions of ET per week, W_{\max} did not increase (-1, -1 and 2 %, respectively) more than $1 \times \%TE$ (4 %) and hence they were considered as CRF non-responders. Afterwards, CRF non-responders accomplished a second ET period including 2 x 60 min additional sessions per week of otherwise identical training. After this additional training period CRF non-response was no longer present in any individual. This unequivocally demonstrates that CRF non-response was not inexorably predetermined in the study subjects and suggests that the potential for CRF improvements might well be present and unveiled with appropriate stimuli in healthy individuals without exception. Such *unanimous* CRF response may also indicate a relatively minor influence of within-subject response variability and measurement error when applying successive higher doses of ET (Bacon *et al.*, 2013; Hecksteden *et al.*, 2015).

Despite our data clearly denote the spurious nature of CRF non-response, a wide interindividual variability in CRF improvements to identical ET dose is plainly illustrated in Figures 1 and 2. Certainly, hereditary factors must be assumed to play a

role on CRF trainability (Bouchard *et al.*, 1999), although family- and large population-based confirmatory/replication studies are needed (Thomis, 2008). In addition, an often overlooked factor is the typical use of some percentage of HR_{max} , VO_{2max} or W_{max} to prescribe exercise intensity, which does not effectively standardize the homeostatic stress caused by ET at the individual level (Meyer *et al.*, 1999; Gaskill *et al.*, 2001; Scharhag-Rosenberger *et al.*, 2010). Therefore, provided a given ET dose, individuals may experience diverse magnitudes of stimuli for adaptive responses. Furthermore, a perfectly identical exercise dose prescription for any individual may prompt distinct homeostatic stress according to its interaction with oscillating factors such as sleep and nutrition, both affecting recovery and readiness to train (Kentta & Hassmen, 1998; Samuels, 2009; Hawley *et al.*, 2011). Collectively considered, a comparable homeostatic stress among individuals involved in ET, as commonly prescribed, seems unlikely (Mann *et al.*, 2014). It follows that a certain degree of interindividual variability in CRF trainability to a fixed ET dose may be generally expected by default due to lack of truly standardized exercise stimuli.

The question arises as to whether variability in CRF response to ET lie upon firm mechanistic bases. ET is commonly associated with a surfeit of phenotypic modifications along the O_2 transport and utilization chain—from lung to mitochondria—, all potentially contributing to VO_{2max} and W_{max} improvements via enhancing convective oxygen delivery to and/or extraction by active tissue (Hawley & Noakes, 1992; Helgerud *et al.*, 2007; Boushel *et al.*, 2011; Lundby & Montero, 2015;

Montero *et al.*, 2015a; Wagner, 2015). The bulk of evidence indicates that haemodynamic and haematological systemic adaptations increasing the capacity to deliver oxygen predominantly explain $\text{VO}_{2\text{max}}$ improvements with short-term (5-11 weeks) ET in healthy individuals (Montero & Diaz-Canestro, 2015; Montero *et al.*, 2015b). Empirical evidence demonstrates that the increase in $\text{VO}_{2\text{max}}$ with 6 weeks of moderate-to-high intensity ET is primarily attributed to augmented circulating RBCV and oxygen-carrying capacity of blood, as reflected by increases in total circulating Hb_{mass} (Bonne *et al.*, 2014; Montero *et al.*, 2015a). In turn, increases in skeletal muscle capillarization and Mito_{VD} , both possibly facilitating oxygen extraction, do not seem to contribute substantially to the increase in $\text{VO}_{2\text{max}}$ after 6 weeks of ET (Montero *et al.*, 2015a). Concurring with the above, in the present study increases (Δ) in Hb_{mass} were the strongest determinants of $\Delta\text{VO}_{2\text{max}}$, which in turn explained most of the variance in $\Delta\text{W}_{\text{max}}$. Yet, approximately half of the variance in $\Delta\text{VO}_{2\text{max}}$ remained unexplained, which could be partially attributed to non-assessed adaptations modulating cardiac output and its distribution (Boushel *et al.*, 2014; Lundby *et al.*, 2016). The parallelism between changes in $\text{VO}_{2\text{max}}$ and underlying phenotypic modifications measured in this study implies the presence of quantitative but not major qualitative differences in either homeostatic stress induced by ET or adaptive capacity on an individual basis. Ultimately, it is worth noticing that the genetic bases of crucial physiological pathways governing convective oxygen delivery have not been identified (Sarzynski *et al.*, 2016).

Another important outcome of this study was the magnitude of increases in CRF at the group level according to ET dose. Increases in W_{\max} with 240 or 300 min were substantial (24 and 32 %) and higher compared with uniformly small W_{\max} changes observed with 60, 120 or 180 min of training per week (1, 5 and 7 %, respectively) (Figure 1). Similar findings were found regarding $VO_{2\max}$ (Figure 3). Part of this gap could be attributed to lower Hb_{\max} in the 300 min group prior to ET ($P = 0.064$) facilitating greater adaptations, albeit baseline values were included as covariates. Regardless, the critical duration of exercise for CRF improvements with moderate-high intensity ET may lie somewhere between $> 180 \leq 240$ min per week for most individuals. This finding concurs with previous reports suggesting to raise the minimum level of exercise dose in physical activity guidelines for healthy adults (Bacon *et al.*, 2013; Ross *et al.*, 2015).

Limitations

Findings were obtained from a sample of healthy young males. Further research will elucidate whether current conclusions can be extrapolated to populations including females, older individuals and/or cardio-metabolic patients. Moreover, this study was designed to dissect the independent contribution of overall ET dose, but not of its components (frequency/length of exercise sessions, total duration, intensity). In addition, $VO_{2\max}$ measurements were not duplicated at baseline and therefore %TE for $VO_{2\max}$ was not calculated. Nonetheless, $VO_{2\max}$ and W_{\max} responses were closely associated and all W_{\max} non-responders increased their $VO_{2\max}$ with higher ET dose

(Figures 3 and 4). Finally, changes in body composition potentially influencing CRF were not assessed.

Conclusion

This study fundamentally challenges the notion of CRF non-response to exercise training. In 78 healthy individuals performing moderate-to-high intensity ET for 6 weeks, the prevalence of CRF non-response, as determined by W_{\max} , gradually declined in individuals exercising 60, 120 or 180 min per week. No vestige of W_{\max} non-response was observed in individuals training 240 or 300 min per week. Following a successive 6-week ET period comprising 120 min of additional ET per week, W_{\max} non-response was universally abolished. Consistent with sound experimental evidence, haematological adaptations in oxygen-carrying capacity underlay $VO_{2\max}$ and thereby W_{\max} (non-)responses to ET. These findings highlight the need to re-evaluate contemporary concepts about human untrainability and exercise prescription.

Table 1. Baseline characteristics of study groups ($n = 78$)

	Exercise sessions per week					ANOVA
	1 ($n=16$)	2 ($n=15$)	3 ($n=14$)	4 ($n=17$)	5 ($n=16$)	
Body weight (kg)	76.6 \pm 5.8	76.8 \pm 4.8	77.7 \pm 5.7	77.8 \pm 8.8	79.8 \pm 5.9	0.654
Hct (%)	43.8 \pm 2.3	43.4 \pm 1.6	43.9 \pm 2.1	43.0 \pm 1.6	43.5 \pm 2.2	0.752
Hb (g·dl ⁻¹)	14.6 \pm 0.7	14.4 \pm 0.6	14.9 \pm 0.7	14.8 \pm 0.6	14.5 \pm 0.8	0.229
Hb _{mass} (g)	847 \pm 84	878 \pm 151	826 \pm 103	841 \pm 113	761 \pm 101	0.064
PV (ml)	3266 \pm 532	3440 \pm 501	3077 \pm 376	3246 \pm 453	2976 \pm 430	0.065
RBCV (ml)	2521 \pm 262	2640 \pm 452	2408 \pm 299	2454 \pm 362	2288 \pm 301	0.070
BV (ml)	5787 \pm 772	6080 \pm 935	5486 \pm 637	5700 \pm 792	5264 \pm 696	0.054
Mito _{VD} (%) ^a	4.62 \pm 1.42	4.80 \pm 1.14	4.89 \pm 1.09	3.86 \pm 0.91	4.32 \pm 1.21	0.169
HR _{max} (bpm)	188 \pm 5	185 \pm 7	187 \pm 7	188 \pm 6	187 \pm 7	0.736
RER _{max}	1.15 \pm 0.05	1.15 \pm 0.09	1.17 \pm 0.06	1.13 \pm 0.05	1.14 \pm 0.07	0.510
VO _{2max} (ml·min ⁻¹)	3214 \pm 507	3412 \pm 736	3376 \pm 438	3449 \pm 514	2956 \pm 435	0.069

VO_{2max} (ml·min ⁻¹ ·kg ⁻¹)	42.4 ± 8.5	44.7 ± 10.7	43.4 ± 4.2	44.5 ± 5.8	37.4 ± 7.5	0.057
W_{max} (W)	269 ± 44	289 ± 66	297 ± 42	266 ± 48	239 ± 40 ³	0.015
W_{max} (W·kg ⁻¹)	3.54 ± 0.70	3.80 ± 0.95	3.82 ± 0.45	3.42 ± 0.48	3.02 ± 0.61 ²³	0.008

BV, blood volume; Hb_{mass}, hemoglobin mass; Hct, haematocrit; HR_{max}, maximal heart rate; Mito_{VD}, mitochondrial volume density; PV, plasma volume; RBCV, red blood cell volume; RER_{max}, maximal respiratory exchange ratio; VO_{2max} , maximal oxygen consumption; W_{max} , maximal power output. Superscript numbers (1, 2, 3, 4, 5) correspond to differences ($P < 0.05$) between groups ('1', '2', '3', '4', '5').

^a Mito_{VD} values were not available in all study subjects (sample size of 15, 14, 11, 15, 14 in groups '1', '2', '3', '4' and '5', respectively).

Table 2. Absolute changes (Δ) after the first exercise training period

	Exercise sessions per week					ANOVA ^a
	1 (n=16)	2 (n=15)	3 (n=14)	4 (n=17)	5 (n=16)	
Δ Body weight (kg)	-0.21±1.26	-0.23±0.94	-0.39±1.96	-1.38±1.96*	2.75±0.98* ¹²³	<0.001
Δ Hct (%)	0.03±1.15	-0.31±1.28	-0.91±2.64	-1.33±1.31*	0.20±1.24 ⁴	0.024
Δ Hb (g·dl ⁻¹)	0.03±0.38	-0.04±0.43	-0.18±0.65	-0.10±0.56	-0.00±0.42	0.919
Δ Hb _{mass} (g)	15.6±39.4	40.0±43.6*	34.3±35.8*	44.9±40.4*	137±51.7* ¹²³⁴	<0.001
Δ PV (ml)	31±294	203±206*	263±386*	55±253	555±250* ¹²⁴	<0.001
Δ RBCV (ml)	28±150	108±129*	105±99*	177±147* ¹	398±158* ¹²³⁴	<0.001
Δ BV (ml)	59±429	311±296*	368±446*	232±371*	953±376* ¹²³⁴	<0.001
Δ Mito _{VD} (%) ^b	0.48±0.27*	0.74±0.43*	1.66±0.50* ¹	2.15±1.18* ¹²	1.92±1.32* ¹²	<0.001
Δ VO_{2max} (ml·min ⁻¹)	27±161	152±178*	156±174*	424±207* ¹²³	707±311* ¹²³⁴	<0.001
Δ VO_{2max} (ml·min ⁻¹ ·kg ⁻¹)					10.49±3.9* ¹²³	
¹)	0.42±2.40	2.19±2.39*	2.47±1.90*	6.50±2.98* ¹²³	⁴	<0.001
Δ W_{max} (W)	3.13±12.65	13.13±17.4*	21.18±12.3*	63.37±24.6* ¹²³	75.75±31.3* ¹²³	<0.001
Δ W_{max} (W·kg ⁻¹)	0.05±0.17	0.19±0.23*	0.30±0.16*	0.92±0.39* ¹²³	1.09±0.40* ¹²³	<0.001

BV, blood volume; Hb_{mass}, hemoglobin mass; Hct, haematocrit; Mito_{VD}, mitochondrial volume density; PV, plasma volume; RBCV, red blood cell volume; VO_{2max} , maximal oxygen consumption; W_{max} , maximal power output; Δ , post minus pre training absolute change.

* $P < 0.05$ post training versus pre training. Superscript numbers (1, 2, 3, 4, 5) correspond to differences ($P < 0.05$) between groups ('1', '2', '3', '4', '5').

^a Baseline values included as covariates.

^b Mito_{VD} values were not available in all study subjects (sample size of 13, 12, 9, 15, 12 in groups '1', '2', '3', '4' and '5', respectively).

Table 3. Multiple linear regression with ΔW_{\max} as the dependent variable

Model	β (95 % CI)	r_{partial}	P	Adjusted R^2	P
1.					
Δ Body weight (kg)	-0.001 (-0.004, 0.001)	-0.137	0.307	0.72	<0.001
Δ Hb _{mass} (g)	0.072 (-0.062, 0.207)	0.142	0.287		
Δ PV (ml)	0.003 (-0.017, 0.022)	0.035	0.797		
Δ Mito _{VD} (%)	2.170 (-1.979, 6.318)	0.139	0.299		
Δ VO _{2max} (ml)	0.080 (0.060, 0.100)	0.738	<0.001		
2. (including RBCV instead of Hb _{mass})					
Δ Body weight (kg)	-0.002 (-0.005, 0.001)	-0.158	0.235	0.72	<0.001
Δ RBCV (ml)	0.023 (-0.020, 0.067)	0.143	0.283		
Δ PV (ml)	0.023 (-0.020, 0.067)	0.143	0.283		
Δ Mito _{VD} (%)	1.988 (-2.256, 6.232)	0.124	0.352		
Δ VO _{2max} (ml·min ⁻¹)	0.080 (0.061, 0.100)	0.742	<0.001		
3. (including BV instead of Hb _{mass} , PV and RBCV)					
Δ Body weight (kg)	-0.002 (-0.004, 0.001)	-0.166	0.210	0.73	<0.001
Δ BV (ml)	0.008 (-0.003, 0.019)	0.181	0.170		
Δ Mito _{VD} (%)	2.462 (-1.569, 6.492)	0.160	0.226		
Δ VO _{2max} (ml·min ⁻¹)	0.083 (0.065, 0.101)	0.773	<0.001		

β , unstandardized regression coefficient with W_{\max} (W)

as outcome; BV, blood volume; CI, confidence interval;

Hb_{mass}, hemoglobin mass; Mito_{VD}, mitochondrial

volume density; PV, plasma volume; RBCV, red blood

cell volume; r_{partial} , partial correlation coefficient; VO_{2max},

maximal oxygen consumption; W_{\max} , maximal power

output; Δ , post minus pre training absolute change.

Δ Hb_{mass}, Δ RBCV and Δ PV were closely correlated with

Δ BV in bivariate analyses ($r > 0.80$, $P < 0.0001$), thus

these variables were appropriately entered into a

regression model in order to avoid high

multicollinearity (VIF >10).

Table 4. Multiple linear regression with Δ VO_{2max} as the dependent variable

Model	β (95 % CI)	r_{partial}	P	Adjusted R^2	P
1.					
Δ Body weight (kg)	-0.021 (-0.051, 0.008)	-0.147	0.153	0.41	<0.001
Δ Hb _{mass} (g)	3.262 (2.076, 4.448)	0.491	<0.001		
Δ PV (ml)	0.015 (-0.019, 0.219)	0.015	0.884		
2. (including RBCV instead of Hb _{mass})					
Δ Body weight (kg)	-0.032 (-0.061, -	-0.218	0.033	0.39	<0.001

	0.002)				
Δ RBCV (ml)	1.002 (0.620, 1.384)	0.473	<0.001		
Δ PV (ml)	-0.031 (-0.253, 0.191)	-0.029	0.884		
3. (including BV instead of Hb _{mass} , PV and RBCV)					
	-0.042 (-0.072, -				
Δ Body weight (kg)	0.012)	-0.273	0.007	0.31	<0.001
Δ BV (ml)	0.330 (0.223, 0.436)	0.531	<0.001		

β , unstandardized regression coefficient with VO_{2max} (ml) as outcome; BV, blood volume; CI, confidence interval; Hb_{mass}, hemoglobin mass; PV, plasma volume; RBCV, red blood cell volume; r_{partial} , partial correlation coefficient; VO_{2max}, maximal oxygen consumption; Δ , post minus pre training absolute change.

Δ Hb_{mass}, Δ RBCV and Δ PV were closely correlated with Δ BV in bivariate analyses ($r > 0.80$, $P < 0.0001$), thus these variables were appropriately entered into a regression model in order to avoid high multicollinearity (VIF >10).

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Additional information

Competing interests

The authors declare no conflict of interest with the present study.

Author contributions

Conception and Design of experiments: C.L.

Collection, analysis and interpretation: C.L., D.M.

Drafting the article or revising it critically for important intellectual content: D.M., C.L.

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FIGURE LEGENDS

Figure 1. Individual percentage changes in maximal power output (W_{\max}) after the first exercise training period in each group. The typical error of measurement (%TE) for W_{\max} measurement is illustrated by the shaded area. Values within this area represent non-response. Non-response was 69 % (11 of 16), 40 % (6 of 15), 29 % (4 of 14), 0 % (0 out of 17) and 0 % (0 out of 16) for '1', '2', '3', '4' and '5' groups, respectively.

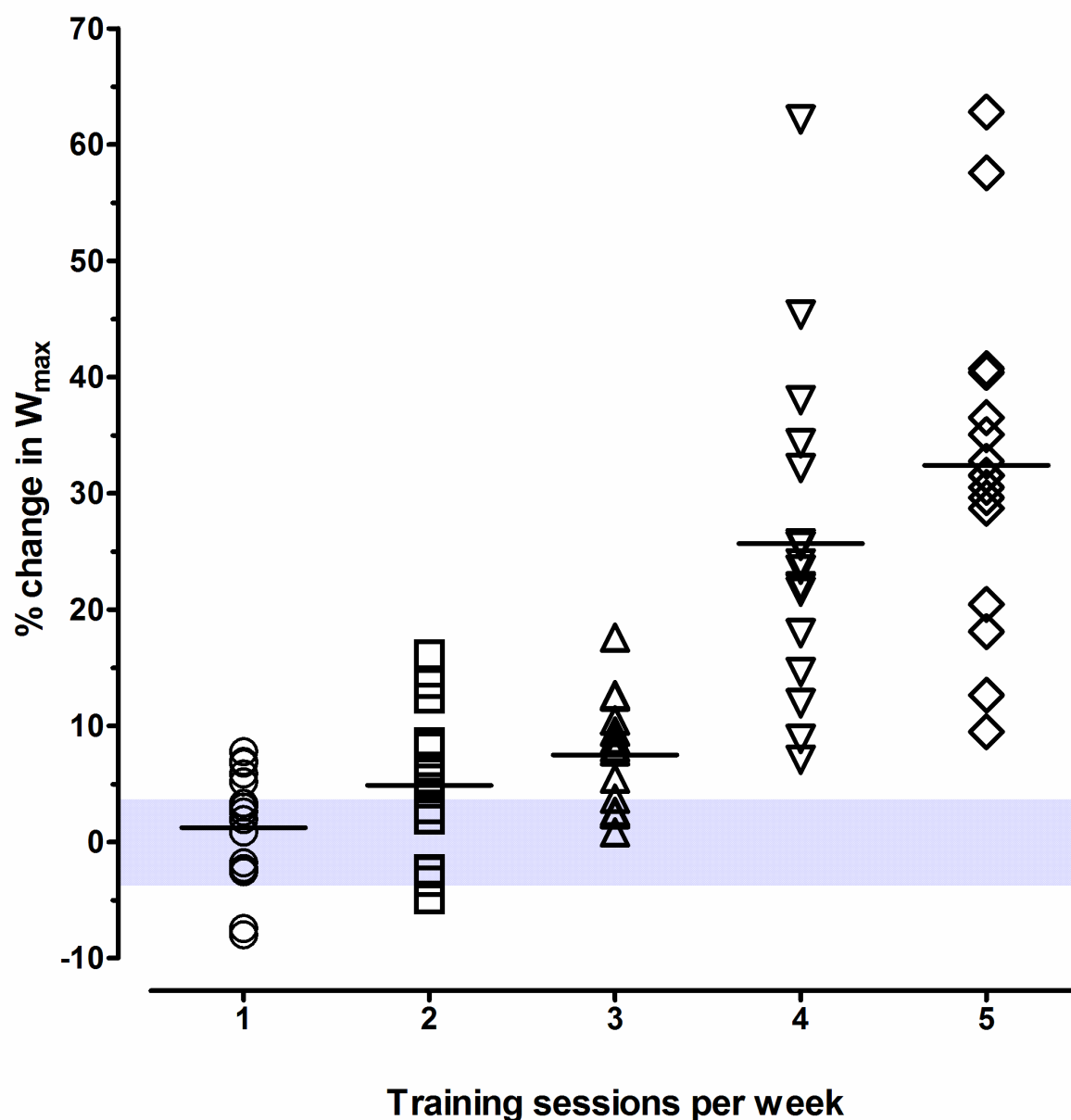


Figure 2. Individual percentage changes in maximal power output (W_{\max}) after the second exercise training period for non-responders in each group. The typical error of measurement (%TE) for W_{\max} measurement is illustrated by the shaded area. Values within this area represent non-response. Non-response was abolished after the second exercise training period in all individuals.

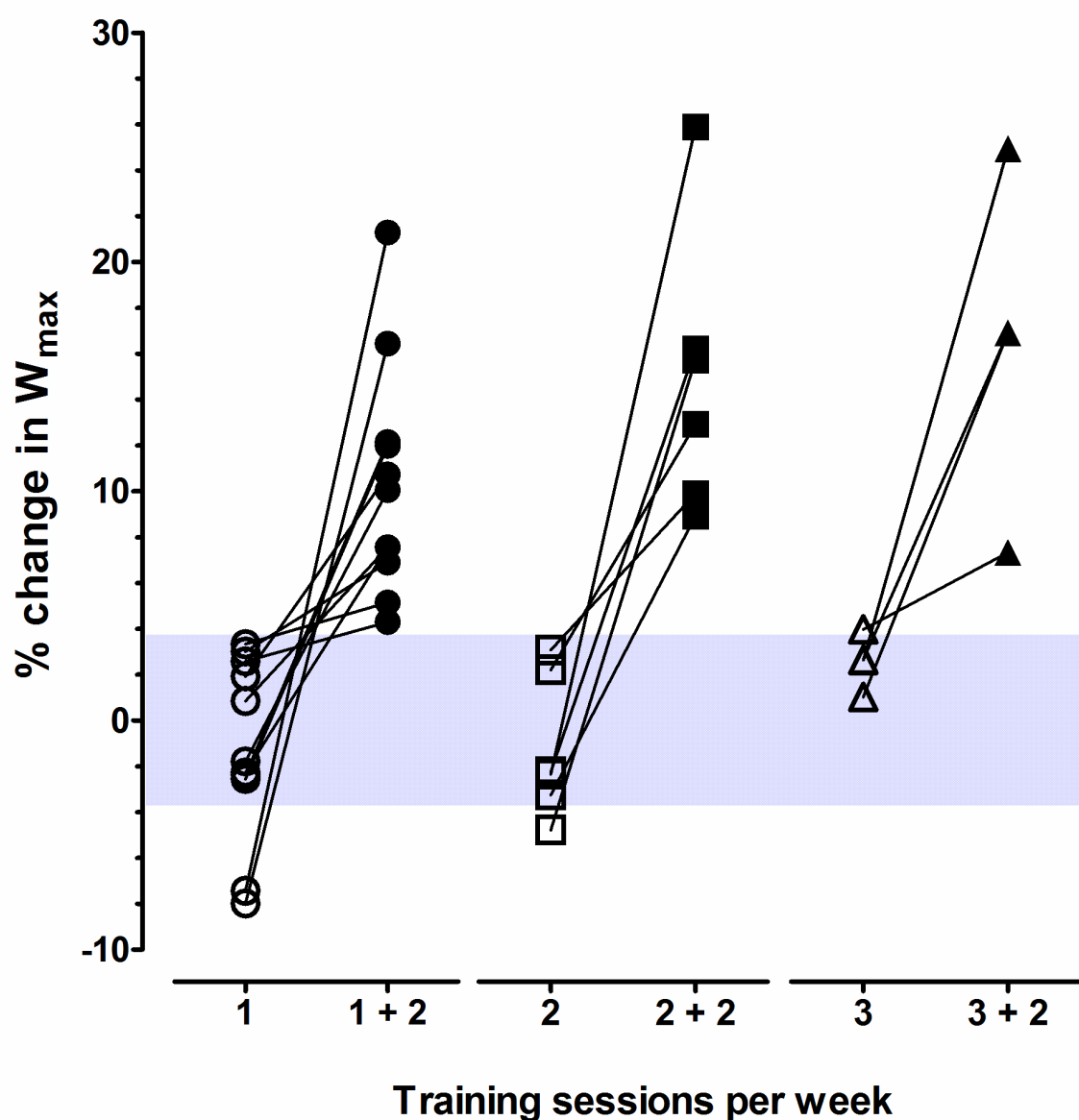


Figure 3. Individual percentage changes in maximal oxygen consumption (VO_{2max}) after the first exercise training period in each group.

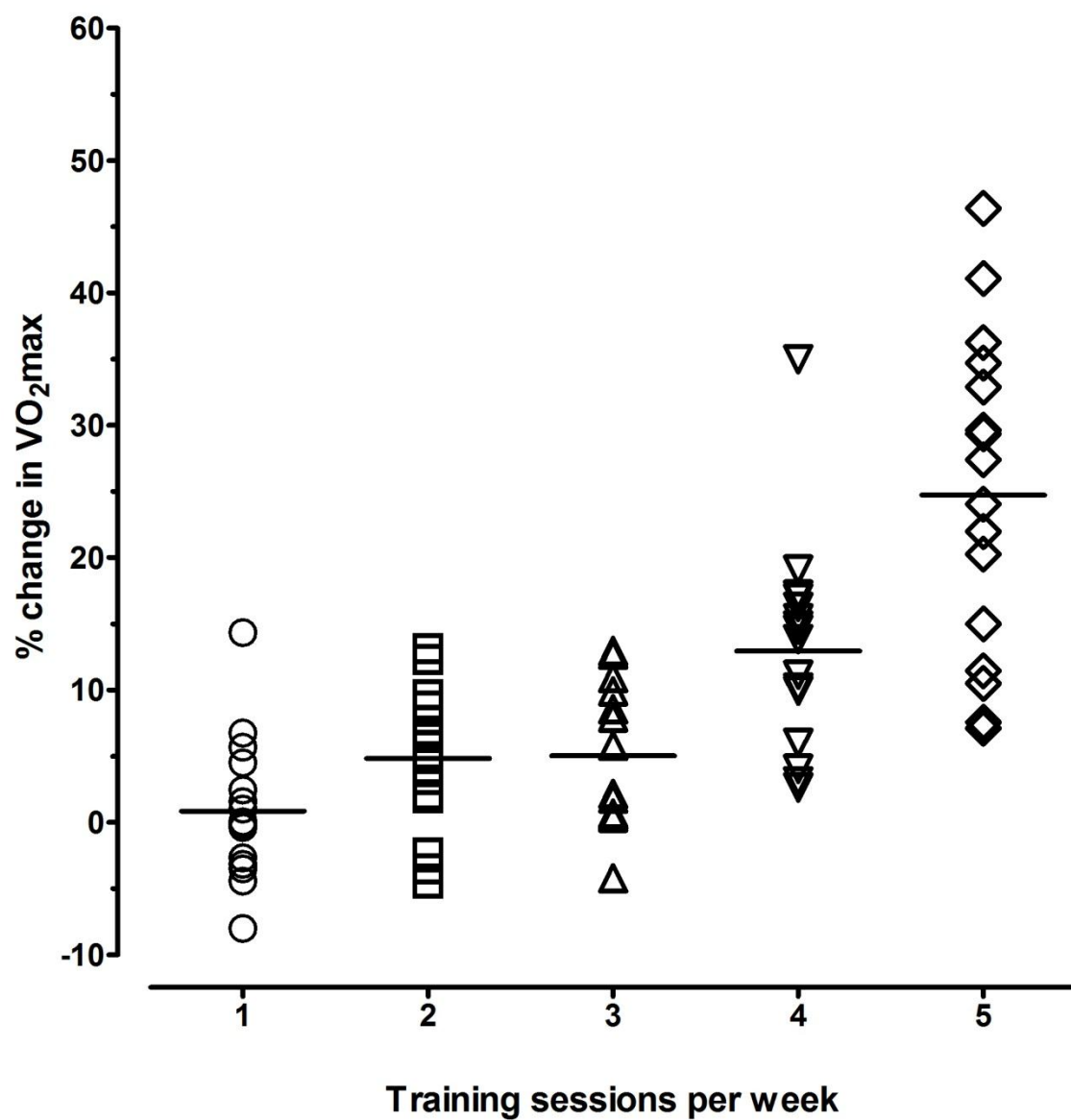


Figure 4. Individual percentage changes in maximal oxygen consumption ($VO_{2\max}$) after the second exercise training period for non-responders (according to changes in maximal power output (W_{\max})) in each group.

